# FURTHER DISCUSSIONS ON THE EFFECTS OF PHASE DIFFERENCE ON THE H-ANTIGEN TRANSDUCTION IN SALMONELLA DIPHASIC STRAINS.

Report by Tetsuo Iino

(June 28, 1955)

In the previous report, the effect of phase differences on the H-antigen transduction in Salmonella was tested. The data are summarized in Table 1 and Table 2. From these results, the hypothesis has been proposed on the mechanism of phase variation; that is, each phase is controled by the different locus, phase 1 by $H_1$ and phase 2 by $H_2$, and $H_2$ suppresses the action of $H_1$, and the phase variation occurres by the mutation of $H_2$ to inactive allele $h_1$. In other words, phase 1 has genotype $H_1h_2$ and phase 2 has genotype $H_1H_2$. In the present paper, the data offered by Dr. Lederberg (Table 3 and Table 4) will be discussed, by comparing them with those of the previous report and with the results deduced from above hypothesis.

Before beginning the discussion of the data, the expected results from the above hypothesis will be considered theoretically, which will be convenient to discuss the data quantitatively. In general, phase 1 cell can transduce $H_1$ or $h_2$ and phase 2 cell can transduce $H_1$ or $H_2$ from phase 1 cell, and $H_1$ or $H_2$ from phase 2 cell in regard to the H-factors, so the frequencies of the phages which carry $H_1$, $H_2$ or $h_2$ in total H-carrying phages are as follows:

$FA(H_1)$----- $l_1(d_1+d_2) = l_1$

$FA(H_2)$----- $l_2d_2$

$FA(h_2)$----- $l_2d_1$

$l_1$ = Probability of $H_1$-incorporation by phage in total H-incorporation. $= \frac{T(H_1)}{T(H_1)+T(H_2)}$

$l_2$ = Probability of $H_2$-incorporation by phage in total H-incorporation.

$l_1+l_2 = 1$

$d_1$ = Frequency of phase 1 cells in donor culture.

$d_2$ = Frequency of phase 2 cells in donor cultures

$d_1+d_2 = 1$

These phages are transduced into recipient cells and produce following four combinations: $\overset{*}{H}_1h_2$, $H_1\overset{*}{H}_2$, $\overset{*}{H}_1H_2$, and $H_1\overset{*}{h}_2$, and the frequency of each type is as follows,

$$\overset{*}{H}_1h_2 \text{ ---- } l_1c_1r_1 \qquad (1)$$

$$H_1\overset{*}{H}_2 \text{ ---- } l_2c_2d_2(r_1+r_2) = l_2c_2d_2 \qquad (2)$$

$$\overset{*}{H}_1H_2 \text{ ---- } l_1c_1r_2 \qquad (3)$$

$$H_1\overset{*}{h}_2 \text{ ---- } l_2c_2d_1(r_1+r_2) = l_2c_2d_1 \qquad (4)$$

$r_1$ = Frequency of phase 1 cells in recipient culture.

$r_2$ = Frequency of phase 2 cells in recipient culture.

$c_1$ = Efficiency of $H_1$-incorporation by recipient cells.

$c_2$ = Efficiency of $H_2$-incorporation by recipient cells.

For the simplification, lc is replaced by t ( transduction coefficient of locus ), then,

$$\overset{*}{H}_1h_2 \text{ ---- } t_1r_1 \qquad (1')$$

$$H_1\overset{*}{H}_2 \text{ ---- } t_2d_2 \qquad (2')$$

$$\overset{*}{H}_1H_2 \text{ ---- } t_1r_2 \qquad (3')$$

$$H_1\overset{*}{h}_2 \text{ ---- } t_2d_2 \qquad (4')$$

When antiserum for the H-antigens of the recipient cells are used as selective agents, type (3) and type (4) are selected away, so the ratio of phase 1 transduced type and phase 2 transduced type is

$$\overset{*}{H}_1 : \overset{*}{H}_2 = t_1r_1 : t_2d_2 \qquad (5).$$

Thus, when antiserums are used as selective agents, the frequency of the transduction of phase 1 increases with the increase of the frequency of the phase 1 cells in recipient culture regardless the frequency in donor cultures, whereas the frequency of the transduction of phase 2 increases with the increase of the phase 2 cells in donor cultures regardless the frequency in recipient culture. The transduced types, when donor, recipient or both contain only one of alternative phases, are shown in Table 5.

Now, we shall turn back to the experimental results. The data shown in table 3 coingide well with results deduced from the hypothesis. Only one contradiction is the appearance of thec2 $H_2$-transduced types in phase 1 -x phase 1 combination. As indicated by formula (5) and Table 5, $H_2$-transduced type appears only when donor contains phase 2 cell, so the possible explanation of this discrepancy may be the contamination of phase 2 during the course of preparation of phase 1-lysate by the phase variation ($h_2$ to $H_2$). The same consequences have been observed also in the phase 1 -x phase 2 and phase 2 -x phase 2 combinations in Table 1, though the direction of the variation is reversed. These few contaminations are very liable to occur as the mutation rates between each phase are very high and the preparation of the lysate requires the growth of the bacterial cells until the numbers which allow the occurrence of the mutation $H_2 \rightleftarrows h_2$.

The experiments shown in Table 2 and Table 4 were performed with same donor (TM2, i:1,2) and recipient (Sal. abony, b:enx) strain but with different lysates and cultures. The results of phase 1 -x mixed phase coincide with each other and also with the theoretical expectation (Table 5). One phase 2 transduced type in the combination of phase 1 -x mixed phase in Table 4 may be explained as the results of the mutation from phase 1 to phase 2 during the preparation of lysate.

In the results of phase 2 -x mixed phase, marked confliction is found out. According to the theoretical expectation, phase 1-transduced type and phase 2-transduced type must appear at the rate of $t_1r_1/t_2$. The result indicated in Table 2 is explained as $t_1r_1 = t_2$, and $r_1 = 0.46$ as reported in the previous paper, so $0.46t1 = t_2$. That is, the transduction efficiency of phase 2 is about half of phase 1 locus.

( $t_1/t_2$ can be calculated also from phase 2 -x phase 1 experiment in Table 4, where the ratio of phase 1 transduced type to phase 2 transduced type coincide with the ratio of tl to $t_2$. So,

$t_1/t_2 = 42/11, \qquad 0.25t_1 = t_2.$

Thus the efficiency of transduction of phase 2 locus is about one fourth of the phase 1. )

While Table 4 showes no phase 1 transduced type in the experiment phase 2 -x mixed phase , which is expressed by the following formula,

$$t_1 r_1 = 0 \quad \text{or} \quad t_1 r_1 \ll t_2.$$

as suggested by Dr. Lederberg, $r_1 = 0.5$. So the remained possibility is

$$t_1 \ll t_2$$

This is completely reverse condition with the previous paees in regard to the efficiency of transduction, and it is required to assume the great variability of $t_1/t_2$-ratio to explain these results without contradiction. Thus, it may be most important to test in what extent the $t_1/t_2$-ratio is vaeiable by the experimental condition ( e.g. the concentration of donor or recipient culture, or the difference of the strain ), in order to proceed the discussion about the mechanism of phase variation on the basis of the proposed hypotheiis.

Table 1.

Transductions between single phase cultures of diphasic strains.

Sal. abony ($Fla^+$, b:enx) -x Sal. heidelberg ($Fla^-$, r:1,2).

| Phase of donor | Phase of recipient | Antigen types of $Fla^+$-transformed cells: Unlinked type r:(1,2) | (r):1,2 | Total | Linked type b:(1,2) | (b):1,2 | Total | Total | Ratio of linked transduction |
|---|---|---|---|---|---|---|---|---|---|
| 1 (b) | 1 (r) | 21 | 0 | 21 | 22 | 0 | 22 | 43 | 0.51 |
| 1 (b) | 2 (1,2) | 0 | 7 | 7 | 1 | 42 | 43 | 50 | 0.86 |
| 2 (enx) | 1 (r) | 11 | 0 | 11 | 30 | 0 | 30 | 41 | 0.73 |
| 2 (enx) | 2 (1,2) | 1 | 10 | 11 | 1 | 38 | 39 | 50 | 0.78 |
| Total | | 33 | 17 | 50 | 54 | 80 | 134 | 184 | 0.73 |

$X^2$ (ratio of linked transduction) = 13.69, n = 3

P = 0.01

Table 2.

Transduction of $H_1$ and $H_2$ from single phase culture to mixed phase culture in the diphasic strains ----(1). Transformed cells were selected by anti-i and -1,2 serum. Sal. typhimurium -x Sal. abony.

| Donor | Recipient | No. of the transformed cells i:(enx) | (b):1,2 |
|---|---|---|---|
| TM-2 phase 1 (i) | SW-803 (b:enx) | 19 | 0 |
| TM-2 phase 2 (1,2) | " | 14 | 14 |

Table 3.

Transduction of the H-antigen factors between single phase cultures of diphasic strain (Sal. abony, b:enx -x Sal. typhimurium TM-2, i: 1,2). Transformed cells were selected by anti-i and -1,2 serum.

| Phase of Donor | Phase of Recipient | No. of the transformed cells b:(1,2) | (i):enx |
|---|---|---|---|
| 1 (b) | 1 (i) | 1 0 | 2 |
| 2 (enx) | 1 (i) | 4 2 | 1 1 |
| 1 (b) | 2 (1,2) | 0 | 0 |
| 2 (enx) | 2 (1,2) | 0 | 1 7 |

Table 4.

Transductions of $H_1$ and $H_2$ from single phase culture to mixed phase culture in the diphasic strains ----(2). Transformed cells were selected by anti-i and -1.2 serum. Sal. typhimurium -x Sal. abony.

| Donor | Recipient | No. of the transformed cells i:(enx) | (b):1,2 |
|---|---|---|---|
| TM-2 phase 1 (i) | SW-803 (b:enx) | 3 3 | 1 |
| TM-2 phase 2 (1,2) | " | 0 | 1 2 |

morphase TM 2 ?

Table 5.

The results of H-transduction between diphasic strains, expected from the proposed hypothesis when selected by the antiserum for the antigen of the recipients.

| Phase of donor | Phase of recipient | $d_2$ | $r_1$ | Ratio of $H_1$-transduced type to $H_2$ transduced type $H_1$ : $H_2$ |
|---|---|---|---|---|
| 1 & 2 | 1 & 2 | $0<d_2<1$ | $0<r_1<1$ | $t_1r_1$ : $t_2d_2$ |
| 1 | 1 & 2 | 0 | $0<r_1<1$ | $t_1r_1$ : 0 |
| 2 | 1 & 2 | 1 | $0<r_1<1$ | $t_1r_1$ : $t_2$ |
| 1 & 2 | 1 | $0<d_2<1$ | 1 | $t_1$ : $t_2d_2$ |
| 1 & 2 | 2 | $0<d_2<1$ | 0 | 0 : $t_2d_2$ |
| 1 | 1 | 0 | 1 | $t_1$ : 0 |
| 1 | 2 | 0 | 0 | 0 : 0 |
| 2 | 1 | 1 | 1 | $t_1$ : $t_2$ |
| 2 | 2 | 1 | 0 | 0 : $t_2$ |

$r_1$ = frequency of phase 1 cells in recipient culture.

$d_2$ = frequency of phase 2 cells in donor culture.

$t_1$ = Coefficient of transduction of $H_1$ locus.

$t_2$ = Coefficient of transduction of $H_2$ locus.